Effects of Oil Based Drilling Mud Cuttings on Winter Flounder *(Pseudopleuronectes americanus)*: Absence of Acute Toxicity or Mixed Function Oxidase Induction

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EFFECTS OF OIL BASED DRILLING MUD CUTTINGS ON WINTER FLOUNDER (Pseudopleuronectes americanus): ABSENCE OF ACUTE TOXICITY OR MIXED FUNCTION OXIDASE INDUCTION

by

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Abstract

Addison, R.F., Doe, K., and Edwards, A. 1984. Effects of oil based drilling mud cuttings on winter flounder (Pseudopleuronectes americanus): absence of acute toxicity or mixed fuction oxidase induction. Can. Tech. Rep. Fish. Aquat. Sci. 1307: 19 p.

At the request of the Environmental Co-ordinating Committee of the Canada-Nova Scotia Offshore Oil and Gas Board, the effects of two samples of oil based drilling mud cuttings (discharged to the sea from an East Coast offshore well) on winter flounder (Pseudopleuronectes americanus) were tested.

No lethality was observed after 96h or after seven days exposure to 100% cuttings as a bottom substrate (40 L cuttings: 120 L water) during static tests with either batch of cuttings. Prolonged static exposures over 24 days resulted in two deaths in the control group, and one in an experimentally exposed group (six fish per treatment).

Sub-lethal assays based on mixed function oxidase measurements showed that exposure of flounder to cuttings over 7 or 24 d resulted in no induction of MFO enzymes, nor in the cytochromes associated with them.

Infra-red analyses of hydrocarbons in water showed that maximum concentrations were around 12 $mg \cdot L^{-1}$; most of the components were aliphatic hydrocarbons.

The results show that cuttings from the use of low-aromatic oil based drilling muds have no lethal or sub-lethal effects on winter flounder. Even in this "worst case" scenario of prolonged static exposures, no untoward effects were observed on winter flounder, a species which shows MFO induction in the presence of other hydrocarbons.

Résumé

Addison, R.F., Doe, K., and Edwards, A. 1984. Effects of oil based drilling mud cuttings on winter flounder (Pseudopleuronectes americanus): absence of acute toxicity or mixed function oxidase induction. Can. Tech. Rep. Fish. Aquat. sci. 1307: 19 p.

A la demandu du Environmental Co-ordinating Committee du Canada-Nova Scotia Offshore Oil and Gas Board, on a étudié les effets de deux échantillons de débris de boue de forage contenant du pétrole (rejetés dans l'océan par un puits au large de la côte est) sur la plie rouge (Pseudopleuronectes americanus).

Aucune mortalité n'a été observée après 96 h ou 7 jours d'exposition à un fond composé à 100% de débris (40 L de débris et 120 L d'eau) au cours d'essais statique avec chaque échantillon de débris. Après des expositions statiques prolongées de plus de 24 jours, on a relevé 2 cas de mortalité dans le groupe témoin et un dans un groupe exposé expérimentalement (six poissons par traitement).

Des épreuves subletales basées sur des mesures des oxydases à function multiple ont révélé que l'exposition de la plie rouge à des débris de forage pendant plus de 7 or 24 jours n'entrainait aucune induction d'enzymes MFO, pas plus que dans les cytochromes associés.

L'analyse infrarouge des hydrocarbures dans l'eau a révélé que les concentrations maximales s'élevaient a 12 $mg \cdot L^{-1}$ environ et que la plupart des composantes étaient des hydrocarbures aliphatiques.

Les résultats ont montré que les débris de boue de forage contenant des hydrocarbures pauvres en aromatiques n'ont aucun effet létal ou subletal sur la plus rouge. Même evident le scénario le "plus pessimiste" consistant en expositions statiquee prolongées, on n'a observé aucun effet nocif sur la plie rouge, espèce sujette à l'induction de MFO en presence d'autres hydrocarbures.

Introduction

Drilling "muds" are essential to hydrocarbon exploration and production. They function as lubricants and coolants for the drill bit, as vehicles for removal of the cuttings, and perhaps most importantly to counteract the formation pressures encountered (Johnston, 1981). They consist of fluid suspensions of some mineral such as barite with additives such as detergents or emulsifiers, and usually their density can be adjusted by addition of minerals as the formation pressures demand. Muds used offshore in eastern Canada hitherto have been water-based, although oil based muds (OBM) in which the fluid is a hydrocarbon oil of some kind have been available. OBM tend to be more attractive economically than water based muds; although their initial cost is higher, they perform more efficiently, and result in a net saving. Furthermore, the high initial cost provides an incentive to the operator to recover and re-use the OBM; in the long run this may be desirable from the environmental point of view, as it results in fewer discharges.

Previously the use of OBM was discouraged because the older muds were based on diesel oils which were relatively toxic. More modern OBM are much less toxic, and one operator (Shell) has been permitted to use such an OBM in an east coast well (Alma F-67) on an experimental basis. At the request of the Environmental Coordinating Committee of the Canada-Nova Scotia Offshore Oil and Gas Board, BIO staff were asked to evaluate the potential environmental effects of discharges from the use of these OBM, and this report summarises the results of that evaluation.

Use of OBM at the Alma site

Fig. 1 illustrates the process by which OBM are used in the Alma operation. Essentially, the muds are pumped down the drill hole and are recovered along with

cuttings on the platform. The resulting slurry is then shaken and washed, and the muds recovered for re-use. Only the cuttings are discharged over the side. The cuttings are, however, almost certainly contaminated with some quantities of adsorbed hydrocarbons from the OBM and from formation hydrocarbons if these are present.

In the light of this process, we decided not to test the toxicity of the OBM themselves, partly since this is irrelevant to the Alma situation, and partly because some toxicity tests have already been carried out on these muds (Environmental Protection Service, Atlantic Region, unpublished data). Instead, we decided to examine the potential effects of discharged cuttings.

Material and Methods

Cuttings were obtained from the Alma operation (43° 16' 18.29"N, 60° 39' 55.75"W) on two occasions in February 1984 (batch #1) and April 1984 (batch #2). The cuttings represented drill depths of 3120-3165 m and 4600-4690 m, respectively. The OBM used in the operation was based on a Conoco paraffinic oil containing mainly branched and cyclic alkanes, mostly in the range C_9 - C_{13}, and containing less than 1% aromatics. Cuttings were collected at the outlet pipe immediately before washing (Fig. 1). They were shipped as a slurry in sealed plastic buckets to BIO for testing. Cuttings were stored at 4°C and mixed thoroughly before testing.

Toxicity tests were carried out using winter flounder (Pseudopleuronectes americanus) trawled from shallow water in St. Margaret's Bay, N.S., and held in running seawater at BIO for less than a month until required for testing. This species was chosen as it is a bottom dweller which buries itself in mud, and this habit would tend to maximise its exposure to the cuttings. In addition, the species

is attractive as a candidate organism for monitoring the biological effects of other hydrocarbon discharges (e.g., Payne and Fancey, 1982). Fish were selected for approximate size (200 - 400 g approx.) but were not sexed before exposures began; as it turned out, most were males.

Exposures were carried out in 300 L fibreglass tanks containing 120 L filtered Bedford Basin seawater, held static but aerated at approx. 1-2 L air·min^{-1}. Cuttings, sand or mixtures of sand and cuttings were added at a volume of 40 L. In the first series of tests (March 13-20, 1984) using batch #1 cuttings, four groups of five fish were used; treatments consisted of (a) control (40 L washed sand), (b) 33% cuttings (13.3 L cuttings and 26.7 L sand), (c) 100% cuttings (40 L cuttings) and (d) 100% cuttings washed with 12 vol. water from a high pressure hose in an attempt to simulate discharge conditions (Fig. 1). Water temperatures during the exposures ranged from 1-5.5^{0}C. Acute toxicities were assessed over a 96h period, but exposures were extended to seven days after which the fish were killed and analysed for indices of MFO induction, to indicate any sub-lethal effects of the treatments.

The second series of tests (May - June, 1984) was carried out similarly except that four groups of six fish were used, and exposures were extended to 24 days. The treatments consisted of (a) control (40 L washed sand), (b) 100% batch #1 cuttings (40 L), (c) 33% batch #2 cuttings (13.3 L sand and 26.7 L cuttings) and (d) 100% batch #2 cuttings (40 L). Water temperatures ranged from 1.0 - 8.4^{0}C.

MFO induction in flounder liver was assessed by measuring microsomal protein content, concentrations of Cytochromes b_5 and P-450, and activities of benzyprene hydroxylase and ethoxyresorufin O-de-ethylase, both of which are induced dramatically by traces of aromatic hydrocarbons (eg. Addison et al., 1982). Liver homogenates were prepared in 1.15% KCl (1:4, w:v); crude homogenates

were centrifuged at 10,000 x g for 20 mins to remove mitochondria and cell debris, and microsomes were collected by centrifuging at 100,000 x g for one hour. The microsomal pellet was resuspended in 1.15% KCl. Cytochromes b_5 and P-450 were measured as described by Omura and Sato (1964); benzpyrene hydroxylase by the method of Nebert and Gelboin (1968) and ethoxyresorufin O-de-ethylase by the method of Burke and Mayer (1974).

Measurements of MFO induction were analysed statistically by t test or by analysis of variance.

Samples of cuttings and of water were taken at the start and end of the experimental exposures for hydrocarbon analysis by infra-red (IR) spectroscopy as described by Conrad et. al (1984).

Results and Discussion

Table 1 summarises the acute toxicity data. No deaths were recorded in any exposure during the first 96h. In the first experiment, exposures were extended to 7 days without any deaths. In the second series of tests, exposures were continued to 24 days, and deaths occurred in the control group (one each at 19 and 23 d) and in the 33% #2 batch (one at 23 d). No deaths occurred in 100% cuttings from either batch #1 or #2. Since control group mortality exceeded that in the cuttings treatment, the deaths cannot be ascribed to treatment. Most probably they were due to build up of ammonia or to infection, which would be aggravated by the long static exposure.

The acute lethality test results can be summarised as showing that 96 h or 7 d LC50's were above 25% by volume of cuttings in static exposures (40 L cuttings in a total of volume of 160 L).

Some apparent differences in the behaviour of fish exposed to cuttings as

compared to those in control groups were noted. Whereas the control fish could bury themselves in the sand, or 33% cuttings, flounder exposed to 100% cuttings did not do so; however, this may have been due to the very sticky nature of the cuttings, which would have prevented burrowing.

This absence of acute toxicity was not surprising, in the light of the IR analyses of oil in water (Table 2, taken from Conrad et. al., 1984). It is worth noting that batch #1 cuttings released more hydrocarbons into the water (up to 12.17 mg $\cdot L^{-1}$) than did batch #2 (up to 1.9 mg$\cdot L^{-1}$). However, it is not surprising that neither of these concentrations was lethal, since aromatic hydrocarbons - to which toxicity is attributed (Moore and Dwyer, 1974; Anderson et al., 1974) - account for less than 1% of the total hydrocarbons in the base oil, and would therefore have represented only 10-100 g$\cdot L^{-1}$ in water. This concentration range is well below that usually associated with lethality caused by aromatic hydrocarbons (e.g., Anderson et al., 1974).

Oil concentrations in the cuttings stayed reasonably constant during the tests and ranged from 6.4 - 10.1% wet wt. (batch #1) and 8.5 - 10.1% wet wt. (batch #2) (Conrad et al., 1984).

Sub-lethal toxicity was assessed by measurements of the induction of (MFO) enzymes in flounder liver; MFO in general has been shown to be a sensitive and early warning of biological effects of certain types of pollutants. Usually, aromatic or chlorinated hydrocarbons will cause an increase in components of the MFO system (for a review, see Addison, 1984) especially in enzyme activities associated with Cytochrome P-448. In the present results (Tables 3 and 4), neither the enzyme activities nor levels of cytochrome P-450 (which is involved in the electron transfer processes catalysed by these enzymes) were significantly increased. Cytochrome b_5 was slightly increased in two treatments during the

prolonged exposures, but since this was the only response associated with MFO induction, and since it did not occur consistently in all exposed groups, we cannot ascribe much significance to it.

In general, MFO enzyme activities were higher in all the groups studied during May and June than those examined in March. This is consistent with seasonal variation of MFO activity in St. Margaret's Bay flounder populations; in these fish, MFO activity is generally low during the spawning period of March-April, but recovers rapidly during May and June (R.F. Addison and A. Edwards, unpublished data). The similarity between the natural behaviour of MFOs in unexposed populations and that in experimentally exposed groups is further evidence that exposure to OBM cuttings has no detectable effect on MFO activity. These data extend, and are consistent with, the absence of acute toxicity, and with the conclusion that no appreciable aromatic hydrocarbons were present. They show that not only were there no acute effects resulting from these cuttings, but that even after prolonged exposures, there were no indications of sub-lethal effects.

In summary, both acute and sub-acute toxicity tests showed that cutting discharges from the use of oil based drilling muds had no effect on winter flounder at concentrations as high as 25% by volume cuttings in water, in static tests. This can probably be considered as a "worst case" scenario, as in the natural environment contaminated water would be continually replaced, and fish would be able to avoid contaminated water and sediment. At least on the basis of these limited data there seems, to be no reason to prohibit the use of such low toxicity OBM, provided reasonable precautions are taken in their management, and in the discharge of wastes resulting from their use (COGLA, 1984). However, benthic invertebrates may be more sensitive than fish to these cuttings (Hutcheson et al. 1984) and further studies on this effect may be desirable.

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TABLE 1

Acute toxicities of oil based drilling mud cuttings in winter flounder (Pseudopleuronectes americanus) static exposures, as described in text

Treatment	Fish dead/Fish exposed		
	4d	7d	24d
Control: sand (40 L) + 120 L water	0/5	0/5	ND[a]
33% #1 cuttings (13.3L) + sand (26.7L) + 120 L water	0/5	0/5	ND
100% #1 cuttings (40 L) + 120 L water	0/5	0/5	ND
100% #1 washed cuttings (40 L) + 120 L water	0/5	0/5	ND
Control: 40 L sand + 120 L water	0/6	0/6	2/6
33% #2 cuttings (13.3L) + sand (26.7L) + 120 L water	0/6	0/6	1/6
100% #2 cuttings (40 L) + 120 L water	0/6	0/6	0/6
100% #1 cuttings (40 L) + 120 L water	0/6	0/6	0/6

[a]ND: Not determined

TABLE 2

Oil concentrations ($mg \cdot L^{-1}$) in water from experimental 4 d and 24 d exposures of flounder (Pseudopleuronectes americanus) to oil based drilling mud cuttings on two occasions (March 1984; May-June 1984) as described in text.

Treatment	Oil Concentration ($mg \cdot L^{-1}$)	
	Start	**End**
	(12 March)	**(16–17 March)**
Control	0.03	0.25
100% batch #1 cuttings	0.93	8.01
100% batch #1 cuttings, washed	1.56	5.22
	(11 May)	**(4 June)**
Control	1.52	0.43
100% batch #1 cuttings	3.53	12.17
100% batch #2 cuttings	1.88	0.38

TABLE 3

Indices of MFO Induction in Flounder (Pseudopleuronectes americanus) exposed for 7 days to oil-based drilling mud cuttings (batch #1) as described in text. All data as mean ± s.d. (n=5 throughout)

Variable: Treatment	Fish wt. (g)	Liver % Body wt.	Sex	Microsomal protein ($mg \cdot g^{-1}$ liver)	Cyt. b_5 (nmoles·mg protein^{-1})	Cyt. P-450 (nmoles·mg protein^{-1})	BPOH[a] (pmol·mg protein$^{-1}\cdot min^{-1}$)	EROD[b] (nmol·mg protein$^{-1}\cdot min^{-1}$)
Control	294±101	1.04±0.54	4M,1F	12.1±2.0	0.10±0.04	0.23±0.09	29.6±20.4	0.34±0.21
33% batch #1	307±52	0.90±0.11	5M	14.2±3.13	0.13±0.02	0.26±0.04	33.3±9.70.	0.42±0.32
100% batch #1	372±28	1.00±0.24	4M,1F	10.9±1.8	0.21±0.10	0.23±0.04	35.7±11.2	0.33±0.15
100% batch #1, washed	347±66	0.94±0.13	4M,1F	12.2±2.9	0.19±0.06	0.19±0.08	48.2±39.9	0.16±0.10

[a]BPOH : benzypyrene hydroxylase

[b]EROD : ethoxyresorufin O-de-ethylase

TABLE 4

Indices of MFO Induction in Winter Flounder (Pseudopleuronectes americanus) exposed for 24d to oil based drilling mud cuttings (batch #1 and #2) as described in text. All data as mean ± s.d.

Variable: Treatment	Fish Wt. (g)	Liver % body wt.	Sex	Microsomal protein ($mg \cdot g^{-1}$ liver)	Cyt. b_5 (nmoles·mg protein^{-1})	Cyt. P-450 (nmoles·mg protein^{-1})	BPOH[a] (pmol·mg protein^{-1}·min^{-1})	EROD[b] (nmol·mg protein^{-1}·min^{-1})
Control (n=4)	310±28	0.83±0.12	2M 2F	10.3±2.5	0.16±0.08	0.16±0.10	48.2±30.7	0.93±1.00
100% batch #1 (n=6)	277±37	0.78±0.13	6M	11.7±2.1	0.39±0.10*	0.28±0.03	44.5±6.9	1.00±0.26
33% batch #2 (n=6)	327±73	0.91±0.26	5M 1F	14.2±3.5	0.21±0.08	0.29±0.13	49.5±1.06	1.14±0.06
100% batch #2 (n=4)	316±14	0.94±0.13	1M 3F	12.0±1.7	0.28±0.09*	0.26±0.08	36.9±9.3	1.23±0.48

*Significantly different from control value ($P < 0.05$)

[a]BPOH: Benzpyrene hydroxylase

[b]EROD: Ethoxyresorufin O-de-ethylase

Figure 1 Schematic diagram of processing of oil based drilling muds at the Alma F67 well site (Courtesy Shell Canada Resources, Ltd.)